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Anti-*Candida* Activity of Fluoxetine Alone and Combined with Fluconazole: a Synergistic Action against Fluconazole-Resistant Strains

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The purpose of this work was to determine the antimicrobial activity of fluoxetine, alone and combined with fluconazole, against 29 *Candida* strains isolated from patients with vulvovaginal candidiasis. MIC and minimum lethal concentration values ranged from 9.8 to 625 μg/ml for all strains tested. The combination of fluconazole with fluoxetine resulted in synergistic activity against six *Candida* strains, with fractional inhibitory index (FIX) values between 0.15 and 0.31. An indifferent effect was found for the remaining strains, with FIX values between 0.63 and 1.

Within the most common infections caused by *Candida* spp., vulvovaginal candidiasis (VVC) has been estimated to affect about 75% of all women at least once in their lifetime (1–3).

Despite its high incidence, treatment options for VVC are quite limited. The most commonly used drugs are the azoles, with fluconazole the most frequently used one (4).

In recent years, an increase in *Candida* resistance to azoles has been reported, especially regarding non-*albicans Candida* species (5). The presence of mechanisms of resistance make the treatment of VVC a challenge of growing proportions (6–9).

The antifungal activities of antidepressant drugs were first discovered when three patients with chronic VVC were treated with sertraline for premenstrual syndrome and presented no symptoms of candidiasis during the sertraline treatment course (10). In response to this finding, studies have shown intrinsic activities of these agents against fungi (10–12).

Therefore, the main goal of this study was to determine the antimicrobial activity of one selective serotonin reuptake inhibitor drug, fluoxetine, alone and in combination with fluconazole, against different *Candida* spp. in order to expand the knowledge regarding the possible antifungal activity of this drug.

Fluoxetine HCl was kindly provided by Labesfal (Fresenius Kabi Group, Portugal). The stock solution was prepared following the manufacturer's instructions: fluoxetine was dissolved in sterile demineralized water at room temperature to achieve a stock solution of 5,000 μg/ml. Fluconazole (Sigma-Aldrich, Sintra, Portugal) was dissolved in sterile demineralized water to form a stock solution of 512 μg/ml. From the stock solutions, 2-fold serial dilutions were prepared.

Twenty-nine *Candida* spp. were included in this study, corresponding to two American Type Culture Collection strains (*C. albicans* ATCC 10231 and *C. albicans* ATCC 90028) and 27 *Candida* isolates: *C. albicans* ($n = 7$), *C. guillermondii* ($n = 2$), *C. krusei* ($n = 2$), *C. sphaerica* ($n = 1$), *C. tropicalis* ($n = 6$), *C. glabrata* ($n = 4$), *C. parapsilosis* ($n = 4$), and *C. lipolytica* ($n = 1$).

The isolates were characterized to the species level by using both molecular identification and an API 32C apparatus (bioMérieux, Vercieux, France) and were kept frozen in brain heart infusion broth (Difco Laboratories, Detroit, MI) with 5% glycerol (Sigma-Aldrich, Sintra, Portugal) at −70°C until testing. After thawing, the yeast cells were subcultured twice in Sabouraud dex-

TABLE 1 Anti-*Candida* activity of fluoxetine

Yeast strain	Fluoxetine activity	
	MIC (μg/ml)	MLC (μg/ml)
C. albicans ATCC 10231	156	156
C. albicans ATCC 90028	625	625
C. albicans MP14	156	156
C. albicans MP25	156	156
C. albicans MP26	156	156
C. albicans AP25A	312.3	312.3
C. albicans AP26B	312.3	312.3
C. albicans MP27	312.3	312.3
C. albicans MP24	312.3	312.3
C. glabrata MP7	19	39
C. glabrata MP8	19	39
C. glabrata MP28	19	39
C. glabrata MP29	19	39
C. guilliermondii MP1	39	78
C. guilliermondii MP2	312.3	312.3
C. krusei MP16	78	78
C. krusei MP17	312.3	312.3
C. parapsilosis MP12	156	156
C. parapsilosis MP34	625	625
C. parapsilosis MP32	312.3	312.3
C. parapsilosis MP9	312.3	312.3
C. sphaerica AP35B	9.8	9.8
C. tropicalis MP4	156	312.3
C. tropicalis MP5	156	156
C. tropicalis MP37	156	156
C. tropicalis MP38	156	312.3
C. tropicalis MP39	156	312.3
C. tropicalis MP36	312.3	312.3
C. lipolytica MP40	156	312.3

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TABLE 2 Phenotypic classification of *Candida* strains based on susceptibility to fluconazole

Strain	MIC-2[a] of fluconazole (μg/ml)	Phenotype[b]
C. albicans MP24	0.25	S
C. albicans ATCC 10231	0.5	S
C. glabrata MP7	4	S-DD
C. glabrata MP8	16	S-DD
C. glabrata MP28	16	S-DD
C. glabrata MP31	32	S-DD
C. tropicalis MP5	4	S-DD
C. tropicalis MP37	8	R
C. albicans MP25	32	R
C. parapsilosis MP9	64	R
C. krusei MP17	—[c]	R
C. krusei MP16	—[c]	R

[a] MIC-2, the concentration of drug that caused a 50% reduction in turbidity compared to the growth control.
[b] S, susceptible; S-DD, susceptible dose dependent; R, resistant.
[c] —, intrinsically resistant to fluconazole.

trose agar (Biokar Diagnostics, Beauvais, France) for 24 h at 37°C to assess viability.

The fluoxetine and fluconazole MIC values were determined according to a modified CLSI M27-A3 microdilution reference procedure (see Table 1, below) (13). Growth inhibition was visually evaluated after 24 and 48 h of incubation at 37°C under aerobic conditions. For fluoxetine, the MIC was defined as the lowest concentration of drug that completely prevented yeast growth. For fluconazole, the MIC corresponded to an approximately 50% inhibition in growth, which was determined spectrophotometrically (13).

Determination of the minimal lethal concentration (MLC) was carried out according to the methods of Canton et al. (14). All experiments were performed in duplicate and repeated independently three times.

Drugs combinations were studied by using a two-dimensional checkerboard procedure with the two antifungal agents and 12 *Candida* strains (see Table 2, below) (15). Growth inhibition was visually evaluated after 48 h of incubation at 37°C under aerobic conditions.

The type of interaction between fluoxetine and fluconazole was calculated based on the fractional inhibitory concentration (FIC) and fractional inhibitory index (FIX). Synergistic, indifferent, and antagonist interactions were defined by FIX values of <0.5, 0.5 to 4, and >4, respectively (15).

The antifungal effects of fluoxetine against the 29 tested *Candida* strains are presented in Table 1. Depending on the tested concentrations, the drug showed a fungistatic or fungicidal activity against all tested strains. In fact, the MIC and MLC values matched for the majority of the strains (19 of the 29 strains tested). Interestingly, different MIC values were obtained for different strains from the same species, especially for *C. albicans*, *C. tropicalis*, and *C. parapsilosis*.

The antifungal activities of fluoxetine were previously reported for *C. albicans* and non-*albicans Candida* species (11, 12). The previously reported MICs range from 50 to 200 μg/ml, and these values fall within the range for our results (9.8 to 625 μg/ml). The broader range of MICs reported here is probably related to the higher number of strains included.

The checkerboard technique was used to determine the effects of different concentrations of fluoxetine and fluconazole on the growth of 12 *Candida* strains.

The phenotypic classification of the 12 *Candida* strains with respect to susceptibility to fluconazole was made using the CLSI M27-S4 criteria specific for each *Candida* species (Table 2) (16).

Of the 12 strains enrolled in the test, two were susceptible (S) to fluconazole, with MICs ranging from 0.25 to 0.5 μg/ml. The five resistant (R) strains presented MICs between 8 and 64 μg/ml. The two *C. krusei* isolates were intrinsically resistant to fluconazole, as expected.

Table 3 presents the MICs obtained for the two drugs, both alone and in combination. A synergistic effect was observed for 6 of the 12 tested strains, and 4 of these 6 strains were resistant to fluconazole. An indifferent effect was observed for the remaining 6 strains.

When the drugs were used in combination, the fluconazole MIC for resistance strains decreased up to 64-fold.

The final effect of fluoxetine depends on the susceptibility profile of a given strain to fluconazole, since a synergistic effect was more evident in the fluconazole-resistant strains. Still, a markedly indifferent effect was evident in the susceptible strains.

TABLE 3 Checkerboard assay of the effects of fluoxetine and fluconazole alone and in combination against *Candida* strains[a]

	MIC (μg/ml)				FIC (μg/ml)			
Strain	FLO	FLO comb.	FLC	FLC comb.	FLO	FLC	FIX[b]	Effect
C. albicans MP24	156	78	0.25	0.125	0.50	0.50	1	Indifferent
C. albicans ATCC 10231	312.3	156	0.5	0.25	0.50	0.50	1	Indifferent
C. glabrata MP7	9.8	4.9	4	2	0.50	0.50	1	Indifferent
C. tropicalis MP5	156	4.9	4	1	0.03	0.25	0.28	Synergistic
C. glabrata MP8	39	4.9	16	8	0.13	0.50	0.65	Indifferent
C. glabrata MP28	156	4.9	16	4	0.03	0.25	0.28	Synergistic
C. tropicalis MP37	312.3	19	8	2	0.06	0.25	0.31	Synergistic
C. glabrata MP31	78	39	32	1	0.50	0.03	0.63	Indifferent
C. albicans MP25	156	19	32	1	0.12	0.03	0.15	Synergistic
C. parapsilosis MP9	39	9.8	64	4	0.25	0.06	0.31	Synergistic
C. krusei MP17	19	19	—[c]	0.12	1	0.001	≅1	Indifferent
C. krusei MP16	39	9.8	—[c]	8	0.25	0.06	0.31	Synergistic

[a] FLO, fluoxetine; FLC, fluconazole; comb., combination of the two drugs.
[b] The FIX is the sum of the FIC for FLU and that for FLO.
[c] —, intrinsically resistant to fluconazole. The FIC and FIX values were obtained by using a MIC of 128 μg/ml (the result obtained with the checkerboard technique).

Since the concentrations of these drugs in vaginal fluid have not yet been defined, we cannot assume their potential antifungal effects *in vivo*. However, considering the common plasma drug concentration of 200 ng/ml for fluoxetine (17), it appears that these values are much lower than the obtained MICs. However, these drugs undergo extensive plasma protein binding (>97%), and this would explain such low plasma drug concentrations. In addition, the drugs can reach concentrations 20 to 40 times higher in some tissues or fluids, for example, the cerebrospinal fluid (3). A topical application directly in the vagina can be assumed to be a desirable therapeutic approach, as it would allow direct introduction of drug quantities that can result in high drug concentrations in this milieu.

The results from this study encourage us to consider a future use of fluoxetine in topical formulations for the treatment of VVC, mainly if combined with azole drugs, especially fluconazole.

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